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A REPORT OF BACTERIOLOGICAL STUDIES OF SEWAGE TREATMENT PROCESSES CARRIED ON AT THE WARDS ISLAND, NEW YORK, SEWAGE TREATMENT PLANT, MADE IN COOPERATION WITH THE CITY OF NEW YORK

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Cincinnati, Ohio

A Report of Bacteriological Studies of Sewage Treatment Processes Carried on at the Wards Island, New York, Sewage Treatment Plant, made in Cooperation with the City of New York. By Principal Bacteriologist C. T. Butterfield.

[1943]

In response to a request from officials of the City of New York, the writer was detailed to proceed on or about January 17, 1943, to New York, N. Y. for temporary duty, to make bacteriological observations on methods of sewage treatment in use there, in cooperation with the City of New York.

Arriving in New York on January 19, 1943, conferences were held with New York officials, Mr. Gould, Mr. Donaldson and Dr. Edwards, the Wards Island Sewage Treatment Plant was visited and arrangements were made for the required space, equipment, material, etc. to carry on the desired bacteriological examinations. On January 20 and 21, 1943, routine samples were collected throughout the sewage treatment processes in use and the examination of these samples was started. Through January 22 to 25, 1943, the follow-up work of the examination of these samples was continued. At this time 66 cultures, 25 of zoogleal bacteria and 41 of coliform bacteria, which had been isolated, were taken back to the Cincinnati laboratory for further study and identification. The data obtained from this study will be presented below under "Results and Discussion".

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Brief Outline of Plant and Plant Processes

The Wards Island Plant consists of primary settling tanks, four batteries of aeration tanks designated A, B, C and D.

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and four batteries of final settling tanks also designated as A, B, C and D, serving the correspondingly designated batteries of aeration tanks. At the time these studies were made and for several prior months batteries A and B had been operated as activated sludge units under the supervision of representatives of the City of New York, while batteries C and D were operated in accordance with the requirements of the Guggenheim Process under the supervision of representatives of the Guggenheim Company. All four batteries were fed by the same primary effluent, the only variation being that small amounts of iron (4.3 and 3.9 p.p.m. as Fe for January 20 and 21, 1943, respectively), were added for the influent to aeration batteries C and D. Additional differences noted in the treatment processes were: (1) a shortened time of flow for aeration batteries C and D, brought about either by increased flow or by the by-passing of some tanks in each battery, (2) a marked reduction in the amount of air supplied to aeration batteries C and D, and (3) a variation in the amount of sludge returned to each battery. The latter variation is reflected in the suspended solids results which are presented along with the bacteriological data in Table No. 1.

Samples Collected

On each of the days indicated, January 20 and 21, 1943, samples were collected from (1) the primary effluent, (2) the four aeration batteries A, B, C and D, obtaining an aliquot portion from the discharge end of each tank in service in each battery for a composite sample for each battery, and (3) similarly collected and composited samples of the final effluent from each

battery of final settling tanks. The calculated time of flow for each unit was ascertained from plant representatives and all samples were collected in chronological order so that theoretically, at least, each portion collected, as it passed through the system, was representative of the same original sewage as it had entered the plant. Thus the sampling schedule in this study covered an 8 to a 10 hour period each day.

Rationale and Method of Examination, and Results

Three types of bacteria were of interest in this study; (a) the zoogleal bacteria which have been designated by Butterfield and Wattie[1] as the active agents in sewage purification by biological processes, (b) the relative numbers of bacteria of the coliform group as these are the organisms of sewage and of sewage plant effluents which are the criterion for judging the quality of waters, and (c) the aerogenes section of the coliform group is of interest as Phelps and Bovan[2] have indicated that these bacteria may be the active bacterial agents in sewage purification by the Guggenheim Process. Thus, the relative numbers of each of these three types of bacteria present in samples from these sewage treatment processes are quite significant. If the bacteria of either type are markedly predominant it is fairly definite evidence that the predominating type is the active agent in that particular purification process. On the other hand, if the coliform group, or the aerogenes section of this group, are predominant and particularly if they are more numerous in the final effluent than they are in the primary effluent then the stream receiving such an effluent would be of apparently poorer

quality than if the sewage had been added untreated.

Examinations to determine the number of zoogleal bacteria present were limited to samples of the aeration tank mixtures and to samples of final effluent which appeared to contain marked amounts of suspended solids. These samples were treated and their examination was carried out in accordance with Method No. 2 as described by Butterfield and Wattie[1] and more fully by Wattie.[3] That is, immediately before examination diluted portions of sample were shaken with glass beads to break up flocs and disperse bacteria* and the dispersed sample thus obtained was immediately diluted and planted in serial dilution in nutrient lactose broth. Three tubes were planted of each dilution and sufficient dilutions (from 0.01 ml. to 0.0000000001 ml. in some samples) were carried to insure positive growth in the lower dilution and sterile tubes in the higher dilutions. Organisms present in the highest dilutions showing growth were isolated and were considered to represent the predominant type of bacterium in the sample. Transfers of these cultures were taken to the Stream Pollution Investigations Laboratory at Cincinnati, Ohio, where they were subjected to further tests by Associate Bacteriologist

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(*) It should be noted here, as it is in the references cited, that this attempt to disperse the bacteria in the zoogleal flocs is not complete. It is doubted if dispersions of more than 25 per cent of the massed bacteria are ever obtained. It is estimated on the basis of microscopic observations which have been made that the method used produces a dispersion of from 5 to 25 per cent of the clumped bacteria. Thus the counts of zoogleal bacteria reported probably represent only 1/4 to 1/20 of the actual number present.

Elsie Wattie. All of the bacteria listed in Table No. 1 as "Activated Sludge Zoogleal Bacteria" were shown by these tests to produce in liquid media (synthetic sewage or sterilized natural sewage or both) massed growths, simulating activated sludge flocs, which were bound together tenaciously enough to remain intact under the continuous agitation of the aeration required to keep the flocs suspended and to maintain aerobic conditions.

Determinations of the number of coliform group bacteria were made by inoculation of portions of sample into lactose broth in the same manner. Incubation of the lactose broth tubes was for 48 hours with readings at 24 and at 48 hours. All high dilution tubes showing gas production in any amount, and all tubes showing gas at 48 hours, having been negative at 24 hours, were transferred to brilliant green bile lactose broth for confirmation. The production of gas in any amount in brilliant green bile lactose broth during 48 hours of incubation was accepted as a confirmed test for members of the coliform group. Results are expressed as most probable numbers per ml. of sample.

It is not possible to make quantitative differential enumerations of the various members of the coliform group when the primary inoculations are made in liquid media and productive solid media for such differentiation are not available. Consequently, it must be noted that the results given for E. coli in the table represent only those few strains of E. coli that managed to develop in the enrichment medium used, which is described below. To overcome this difficulty for the quantitative determina-

tion of the aerogenes section of the group the same dilutions, and the same number of tubes, examined for the coliform group, were planted in Koser's citrate broth as an enrichment medium. (Koser's citrate broth favors the growth of the aerogenes section, and retards the growth of the coli section, of the coliform group). After 48 hours incubation all Koser's citrate tubes which showed any evidence of growth whatever were transferred to brilliant green bile lactose broth and incubated for 48 hours. Tubes showing gas in any amount during this period were streaked on eosin methylene blue agar plates. After 24 hours of incubation colonies on these plates were examined and representatives of each type of colony present were transferred to agar slants. These agar slant cultures were taken to the Cincinnati laboratory where they were repurified and subjected to the necessary tests to differentiate them as belonging to the coli, the aerogenes, or the intermediate sections of the coliform group. Results for each section of the coliform group are expressed in M.P.N's.

One other observation made consisted of estimates of the number of masses (of about 50 microns diameter or more) of zoogleal bacteria per ml. of sample as determined by direct microscopic examination. In this two estimates were made; one by Senior Biologist J. B. Lackey based on counts made on a wet preparation in connection with the plankton enumerations, and the other based on a detailed count of dry, stained preparations at the Cincinnati laboratory. The latter count is necessarily higher as zoogleal masses, uncomplicated by debrii, are not readily visible in an unstained preparation.

The results obtained from these examinations are presented in Table No. 1. The last two columns of Table No. 1 have been calculated from the data of the table to show the ratio of activated sludge zoogleal bacteria to coliforms and to aerogenes bacteria found in the samples. Thus for the sample of aeration mixture, Battery A, of the 20th, the zoogleal bacteria outnumbered the coliforms by 50 to 1, and the aerogenes by 535 to 1; or stated conversely in the sample of aeration mixture, Battery D, of the 20th, there was only one coliform or aerogenes organism per 960 and 10,000, respectively, of zoogleal bacteria present.

(Insert Table No. 1)

While completing these tests on the cultures obtained at the Wards Island Plant, a sample of returned sludge from the sewage treatment plant at Anderson, Indiana, has been made available for study. This plant is operated under the Guggenheim process. Using the same procedures outlined above a careful examination was made of this sample. Coliform bacteria per ml. of this Anderson sample were 93,000 per ml. while the zoogleal bacteria numbers were found to be at least 93,000,000 per ml. Final tests for the aerogenes and intermediate sections of the coliform group have not yet been completed but their numbers will be less than for the coliforms.

Discussion of Results

Reviewing the data of Table No. 1 some observations regarding the items of bacteriological interest may be pointed out:

TABLE NO. 1

RESULTS OF BACTERIOLOGICAL EXAMINATIONS MADE ON SAMPLES, COLLECTED JANUARY 20 AND 21, 1943
FROM WARDS ISLAND, NEW YORK CITY, SEWAGE TREATMENT PLANT

Date of collection	Source of Samples	Suspended Solids	Masses Zoogleal Bacteria per ml.		Activated Sludge Zoogleal Bacteria per ml. *	Coliform Bacteria per ml. as follows:				Ratio Zoogleal bacteria to	
			Wet Preparation	Dry, stained Preparation		Group	B.coli	A.aerogenes	Intermediates	Coliform Z/c	Aerogenes Z/a
		p.p.m.									
1/20/43	Primary Effluent	94	-	-	-	24,000	-	4,300	4,300		
"	Aeration Mix. Batt. A	1490	1120	11,200	2,300,000	46,000	-	4,300	7,500	50	535
"	" " " " B	1750	800	4,600	2,300,000	43,000	-	-	93,000	54	-
"	" " " " C	1240	1200	11,000	23,000,000	240,000	-	23,000	23,000	96	1000
"	" " " " D	540	160	5,600	230,000,000	240,000	-	23,000	43,000	960	10,000
"	Final Effluent, " A	15	-	-	-	430	-	91	230		
"	" " " " B	26	-	-	-	230	-	230	-		
"	" " " " C	61	-	-	230,000	24,000	4300	46,000	4,300	10	5
"	" " " " D	36	-	-	2,300,000	46,000	9300	4,300	-	50	535
1/21/43	Primary Effluent	96	-	-	-	3,600	360	-	-		
"	Aeration Mix. Batt. A	1710	-	-	2,300,000	3,600	910	-	-	640	-
"	" " " " B	1630	-	-	2,300,000	23,000	-	-	3,600	100	-
"	" " " " C	1000	-	-	-	240,000	-	-	9,100	-	-
"	" " " " D	560	-	-	2,300,000	430,000	-	-	43,000	5	-
"	Final Effluent, Batt. A	21	-	-	22,000	430	-	36	73	51	611
"	" " " " B	22	-	-	-	430	91	36	36		
"	" " " " C	55	-	-	-	9,300	2300	720	360		
"	" " " " D	65	-	-	-	24,000	24,000	-	-		

XXXXXXXXXXXXXXXXXXXX
See the following pages for greater detail.
XXXXXXXXXXXXXXXXXXXX

- Indicates observation not made or information not available from tests made.
* This is a minimum, representing probably not more than 1/4 of the actual number. (See text).

TABLE NO. 1

RESULTS OF BACTERIOLOGICAL EXAMINATIONS MADE ON SAMPLES, COLLECTED JANUARY 20 AND 21, 1943 FROM WARDS ISLAND, NEW YORK CITY, SEWAGE TREATMENT PLANT

Date of collection	Source of Samples	Suspended Solids p.p.m.	Masses Zoogleal Bacteria per ml. Wet Preparation	Masses Zoogleal Bacteria per ml. Dry, stained Preparation	Activated Sludge Zoogleal Bacteria per ml. *	Coliform Bacteria per ml. as follows: Group	B.coli	A.aerogenes	Intermediates	Ratio Zoogleal bacteria to Coliform z/c	Ratio Zoogleal bacteria to Aerogenes z/a
1/20/43	Primary Effluent	94	-	-	-	24,000	-	4,300	4,300		
"	Aeration Mix. Batt. A	1490	1120	11,200	2,300,000	46,000	-	4,300	7,500	50	535
"	" " " " B	1750	800	4,600	2,300,000	43,000	-	-	93,000	54	-
"	" " " " C	1240	1200	11,000	23,000,000	240,000	-	23,000	23,000	96	1000
"	" " " " D	540	160	5,600	230,000,000	240,000	-	23,000	43,000	960	10,000
"	Final Effluent, " A	15	-	-	-	430	-	91	230		
"	" " " " B	26	-	-	-	230	-	230	-		
"	" " " " C	61	-	-	230,000	24,000	4300	46,000	4,300	10	5
"	" " " " D	36	-	-	2,300,000	46,000	9300	4,300	-	50	535
1/21/43	Primary Effluent	96	-	-	-	3,600	360	-	-		
"	Aeration Mix. Batt. A	1710	-	-	2,300,000	3,600	910	-	-	640	-
"	" " " " B	1630	-	-	2,300,000	23,000	-	-	3,600	100	-
"	" " " " C	1000	-	-	-	240,000	-	-	9,100	-	-
"	" " " " D	560	-	-	2,300,000	430,000	-	-	43,000	5	-
"	Final Effluent, Batt. A	21	-	-	22,000	430	-	36	73	51	611
"	" " " " B	22	-	-	-	430	91	36	36		
"	" " " " C	55	-	-	-	9,300	2300	720	360		
"	" " " " D	65	-	-	-	24,000	24,000	-	-		

- Indicates observation not made or information not available from tests made.
* This is a minimum, representing probably not more than 1/4 of the actual number. (See text).

1. Considering first the matter of the predominant type of bacterium in the aeration mixtures it is noted that (a) in the average results of 4 samples of aeration mixtures from Batteries A and B (activated sludge) the zoogleal bacteria outnumber the coliforms by 211 to 1, (b) in the average results from 3 samples of aeration mixtures of Batteries C and D (Guggenheim Process) the zoogleal bacteria outnumber the coliforms by approximately the same ratio, 354 to 1, and (c) in the average results from 4 samples of aeration mixtures of Batteries C and D the zoogleal bacteria outnumber the aerogenes by 2880 to 1. Moreover, the numbers of zoogleal bacteria found in the aeration mixtures of Batteries C and D are of the same order of magnitude as have been found in aeration mixtures from a number of activated sludge installations. If these numbers were corrected on a basis of the relative amounts of suspended solids present* (particularly in the case of Battery D), the proportionate numbers of zoogleal bacteria in the results from Batteries C and D would be materially increased. Thus there is very definite evidence that the zoogleal bacteria are decidedly the predominant organisms in the aeration mixtures of Batteries C and D, as well as in A and B. There was no indication of the predominance of any other variety of bacteria as the zone between the highest dilution showing coliforms and the higher dilutions showing no growth as a rule contained zoogleal bacteria only. This zone on the average covered two or three banks of decimal dilutions. Thus the data

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(*) This would be a legitimate correction as zoogleal bacteria are practically always confined in flocs and are included in the suspended solids.

presented, definitely support the assumption that the zoogleal bacteria are the active purification agents in Batteries C and D as they are in A and B.

2. The data of Table No. 1 suggest that the numbers of coliform bacteria may increase slightly in the aeration mixtures of Batteries A and B while they indicate quite definitely a marked increase of these bacteria in the aeration mixtures of C and D. This is observed more readily from Table No. 2 which has been compiled from the data of Table No. 1. The average coliform content of the primary effluents for the two days under study was 13,800 per ml.

(Insert Table No. 2 and No. 3)

The apparent increase of coliforms in the aeration mixtures of A and B (from 13,800 to 28,900) might be overlooked as merely indicating the well-known inaccuracy of coliform determinations based on results from a few samples. However, in the case of the results for Batteries C and D the increase (13,800 to 288,000 per ml.) is too great and too consistent to permit of any other explanation. There is a definite increase in the numbers of coliforms present. While this increase is also indicated for the aerogenes section of the coliform group it is not so marked, representing only about one-fourth the indicated ratio of increase for the entire group. This would suggest that the coli and intermediate sections of the coliform group increase in numbers in Batteries C and D, at least as extensively as do the members of the aerogenes section.

TABLE No. 2

COLIFORM BACTERIA FOUND IN THE AERATION MIXTURES OF BATTERIES A AND B, AND C AND D, OF THE WARDS ISLAND, NEW YORK, PLANT ON JANUARY 20 AND 21, 1943

Date January 1943	Coliforms per ml. in Aeration Batteries	
	A and B	C and D
20	46,000	240,000
20	43,000	240,000
21	3,600	240,000
21	23,000	430,000
Total	115,600	1,150,000
Average	28,900	288,000

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TABLE NO. 3

COLIFORM BACTERIA FOUND IN THE FINAL EFFLUENTS OF BATTERIES A AND B, AND C AND D, OF THE WARDS ISLAND, NEW YORK, PLANT ON JANUARY 20 AND 21, 1943

Date January 1943	Coliforms per ml. in Final Effluent of Batteries	
	A and B	C and D
20	430	24,000
20	230	46,000
21	430	9,300
21	430	24,000
Total	1520	103,300
Average	380	25,800
Percent Remaining	1.3	9.0

3. When the bacteriological data of the final effluents are studied, definite evidence is found that larger numbers of coliform bacteria survive in the effluents of Batteries C and D than in the effluents of A and B. This increased survival is noted more clearly from Table No. 3 which has been compiled from the data of Table No. 1.

The average number of coliforms present in the final effluents of Batteries A and B was 380, and in C and D 25,800 per ml. Expressed on the basis of the percentage remaining of the coliforms which were present in the aeration mixtures, the figure for Batteries A and B is 1.3 per cent, and for C and D 9.0 per cent. Thus the data from the final effluents indicate not only that there is a larger number of coliforms in the final effluents of Batteries C and D as compared with A and B, but also that Batteries C and D are less efficient in removing the coliforms present.

4. A comparison of the average coliform content per ml. of the sewage under treatment (13,800) with the coliform content of the final effluents of Batteries A and B (380) and Batteries C and D (25,800) discloses that while the average for the effluents of Batteries A and B show a marked decrease (2.8 per cent remaining) the average for the effluents of Batteries C and D do not show a decrease but an increase (187 per cent remaining). Thus if the sanitary quality of the waters of the receiving stream were judged by its coliform content, as is the standard practice, the stream receiving the effluents from Batteries C and D would

be considered to be of poorer quality than if it had received the same quantity of the untreated sewage.

It has been suggested that the addition of iron (ferric sulphate) is responsible for this increase in the numbers of coliform bacteria and definite laboratory evidence, using synthetic sewage, has been presented to show that the growth of Aerobacter aerogenes is stimulated by the addition of small amounts of iron. It may well be that other bacteria, including the other members of the coliform group are similarly stimulated. In like manner the same stimulation may have occurred here in natural sewage under conditions of plant operation.

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However, on the basis of the results obtained in the installation under study at this time, other explanations appear to be in order. Thus, for example, this increase in the number of coliforms in the aeration mixtures of Batteries C and D, while definite and marked, may not be any greater than actually occurs in Batteries A and B as the tendency of many bacteria to increase under such conditions is well known. The actual increase in coliform numbers in Batteries A and B may be masked to a great extent by some continuous bacteria-reducing action.

Casual microscopic observations indicated that the numbers of ciliates (known bacteria-eaters) were much less in samples from Batteries C and D. To obtain definite information on this point reference was made to the Memorandum Report of March 11, 1943 of Senior Biologist J. B. Lackey, who was similarly detailed to New York to make a contemporaneous study of the plankton forms in these

sewage purification processes. The data of his Table No. 1 show that the average "Ciliata" content in terms of organisms per ml. for aeration Batteries A and B was 16,060, while the corresponding figure for Batteries C and D was 1220. The average number of ciliates in A and B exceed that in C and D by approximately 15,000 per ml. It is noted also that the other groups of the plankton, particularly the "Rhizopoda" and the "flagellata", some of which may be bacteria-eaters, are also more numerous in Batteries A and B than they are in C and D.

For present purposes the discussion is limited to the consideration of the numbers of ciliates, the known bacteria-eaters. From past studies made in this laboratory, it is known that one of the ciliates, Colpidium colpoda, when using bacteria only as food, consumes per individual generation time approximately 40,000 bacteria of the size of Aerobacter aerogenes. To develop the concept in mind three assumptions are made; (1) that all of ciliates under consideration in this count are of the same relative size and food requirements as Colpidium colpoda (some of them are very much larger), (2) that under the conditions not more than 10 per cent of the ciliates food is made up of bacteria, and (3) that the average generation time of the ciliates is 24 times that of the bacteria. (Based on estimate of 12 hours for ciliates and 30 minutes for bacteria). Under these conditions the presence of the additional number (15,000) of ciliates in the aeration mixtures of Batteries A and B would be responsible for the removal of $\frac{40,000 \times 15,000 \times 10}{100 \times 24} = 2,500,000$ bacteria per ml.

This calculation necessarily is exceedingly crude as one does not know for instance whether under these conditions the ciliates utilize bacterial cells as one per cent, or as 99 per cent, of their required food. However, it is known that the ciliates as a class are avid bacteria-eaters and this calculation is presented to point out the probable influence of this marked difference in the number of ciliates in the two sewage purification processes.

As the majority of the ciliates depend on a "sweeping" action of the adjacent water to draw in their food it would be expected that free bacterial cells in suspension, outside the flocs, would constitute the major portion of any bacterial population consumed. The coliform bacteria which normally grow dispersed throughout the medium, not in flocs, would be easily accessible to such a method of ingestion.

SUMMARY

The bacteriological results obtained from a brief study of the treatment of sewage by the activated sludge process and by the Guggenheim process of sewage purification are presented. It was found that:

1. The zoogleal bacteria, previously described as the active agents in sewage purification by the activated sludge and trickling filter processes, were the predominant species of bacteria in two plants treating sewage by the Guggenheim Process.

2. The apparent increase of coliform bacteria in the aeration mixtures of sewage treated by the Guggenheim Process is much

greater than when treated by the activated sludge process.

3. The final effluents from a plant treating sewage by the Guggenheim Process contains many more coliform bacteria than from a plant treating the same sewage by the activated sludge process.

4. In the installation under study the final effluent from a plant treating sewage by the Guggenheim Process contained more coliform bacteria than the untreated sewage.

A theory is advanced to explain the apparent increase in the number of bacteria of the coliform group.

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